Biosecurity Plan

Roaring River Hatchery

2009

Hatchery Manager 01/12/2010 Date

Aquatic Animal Health Specialist 1/12/2010 Date

Hatchery Systems Manager 01/12/2010 Date

Introduction

Roaring River Hatchery is one of the oldest fish hatcheries in Missouri first built in 1910. In 1938 the Civilian Conservation Corps (CCC) began remodeling it. They added hand-quarried stone buildings and raceways, many of which still stand today. Additional raceways were added in 1961 along with a pump to supplement water flow from the river when the spring flow is low. More improvements were made from 2005-2008, which included a new intake, sealing of leaks in the dam that retains water to feed the hatchery, new water control gates, and a liquid oxygen system. There are a total of 40 outside raceways and 12 inside troughs or raceways (Figures 1-3).

The hatchery's main purpose is to supply trout to Roaring River State Park daily from March through October for a put-and-take fishery and to supply trout for the Roaring River, Capps Creek, and Hickory Creek white ribbon trout management areas.

Annually, around 200,000 rainbow trout are produced at Roaring River. Other hatcheries contribute around 100,000 rainbow trout that are 5 to 12 inches. Most fish received are 8 inches or less and are grown to 12-12.9 inches for stocking. In addition, 500-1,500 brown trout are also received from Shepherd of the Hills Hatchery and are stocked in the Roaring River White Ribbon Trout Management Area or in the park.

Roaring River Spring, the state's 20th largest spring, is the source of water for the hatchery. The spring produces an average of 20.4 million gallons per day (mgd) with a range of 5-144 mgd. Under full production the hatchery requires 12-14 mgd. An electric pump can supply the hatchery with as much as 3 mgd of water recirculated from the stream.

Background and Purpose of Plan

The purpose of this plan is to identify and minimize possibilities for disease transfer or introduction of nuisance species in the watershed. It will provide a protocol for staff to follow during operation of the fish hatchery.

Although not now present in the watershed, zebra mussels and parasitic copepods do exist in watersheds associated with other MDC hatcheries and there is the risk that these nuisance species may be inadvertently transferred to Roaring River. Roaring River fish have had a number of serious, recurring infections of enteric red mouth (*Yersinia ruckeri).* Other pathogens appearing from time to time include furunculosis (*Aeromonas salmonicida*), bacterial gill disease (*Flavobacterium branchiophilum*), columnaris (*Flavobacterium columnare),* fin rot and Aeromonad septicemia (*Aeromonas complex* and *Pseudomonas spp.),* bacterial kidney disease (*Renibacterium salmoninarum)* and coldwater disease

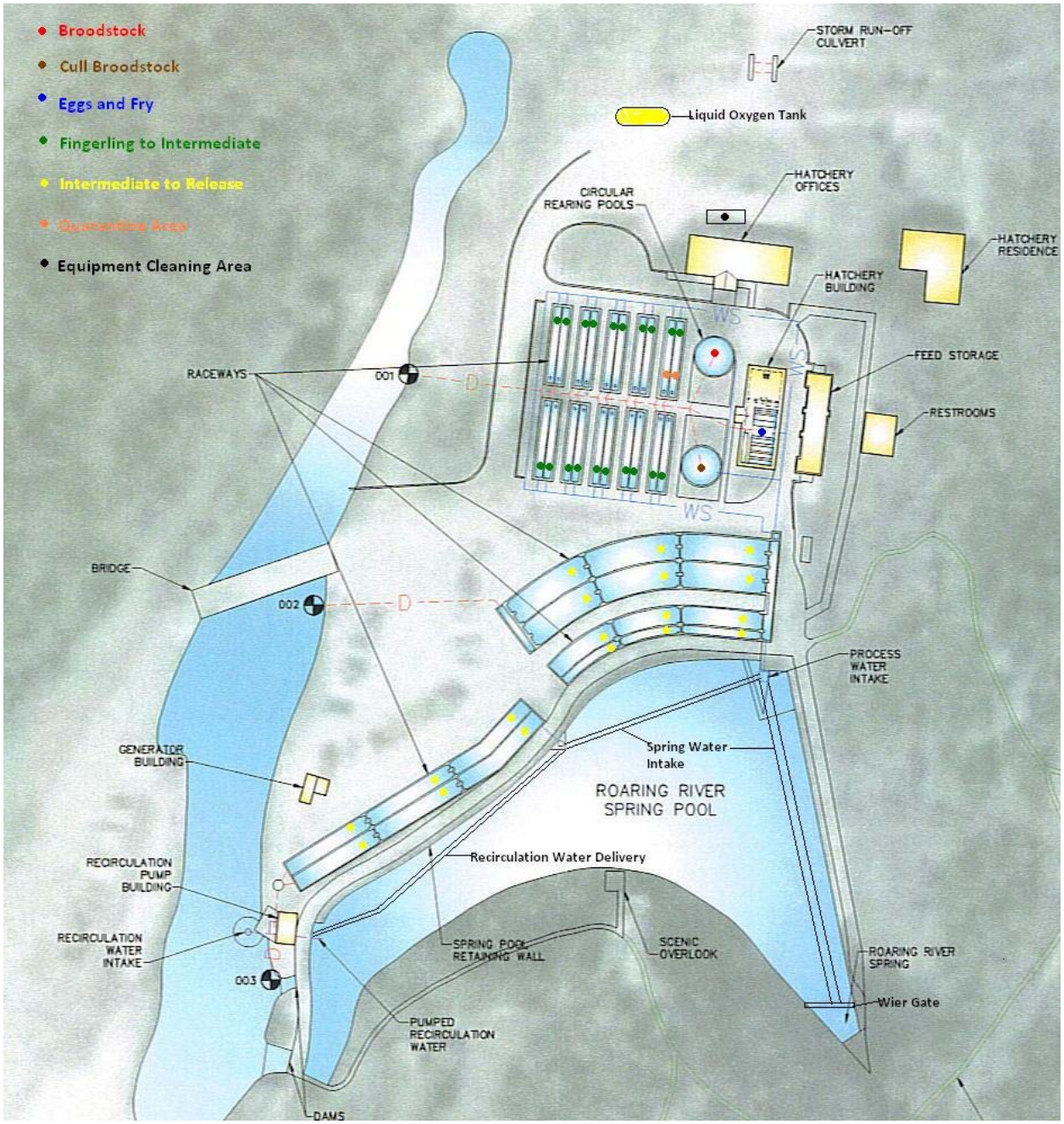


Figure 1. Roaring River Hatchery layout.

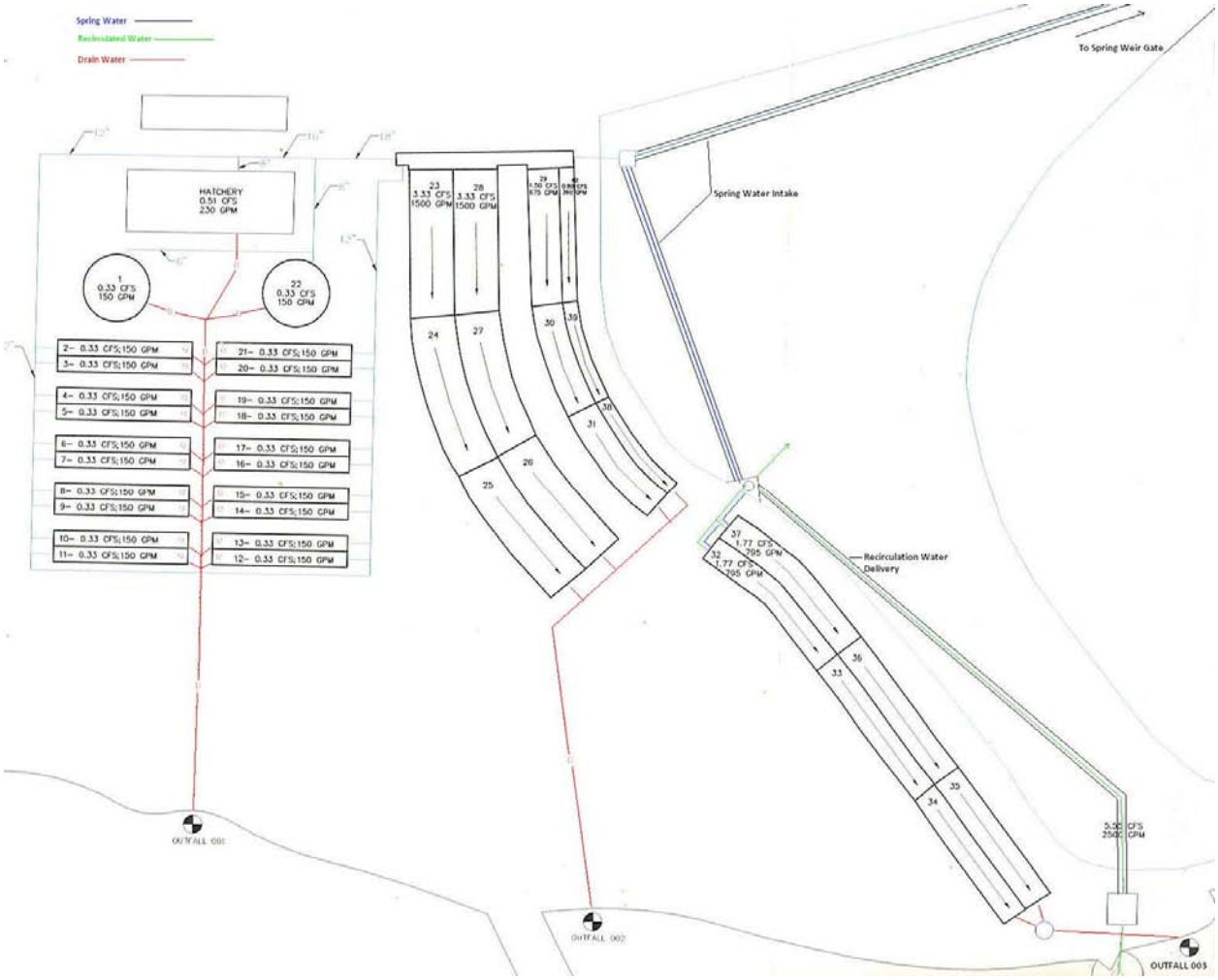


Figure 2. Water flow direction and type.

(Flavobacterium psychrophilum). Protozoan disease agents in order of importance include:*Ichthyoboda (previously known as Costia)*, *Trichodina*, *Ichthyophthirus multifilis (ich*), C*hilodonella, Ambiphrya* and *Epistylis*. Occasionally, monogenetic trematodes such as *Gyrodactyulus*, become numerous on some groups of fish and cause mortality. The fungus *Saprolegnia* periodically causes mortality in eggs and very young fry. Spring water supersaturated with nitrogen gas is a frequent problem for young fry and fingerlings. An ultraviolet (UV) light system disinfects water going into the hatchery building where eggs are hatched and fry are reared. The rest of the hatchery's water comes directly from the spring. The biggest constraint in Roaring River Hatchery's biosecurity measures is that the effluent discharges directly into Roaring River and ultimately into Table Rock Lake. This situation restricts most disinfectants from being added to the water.

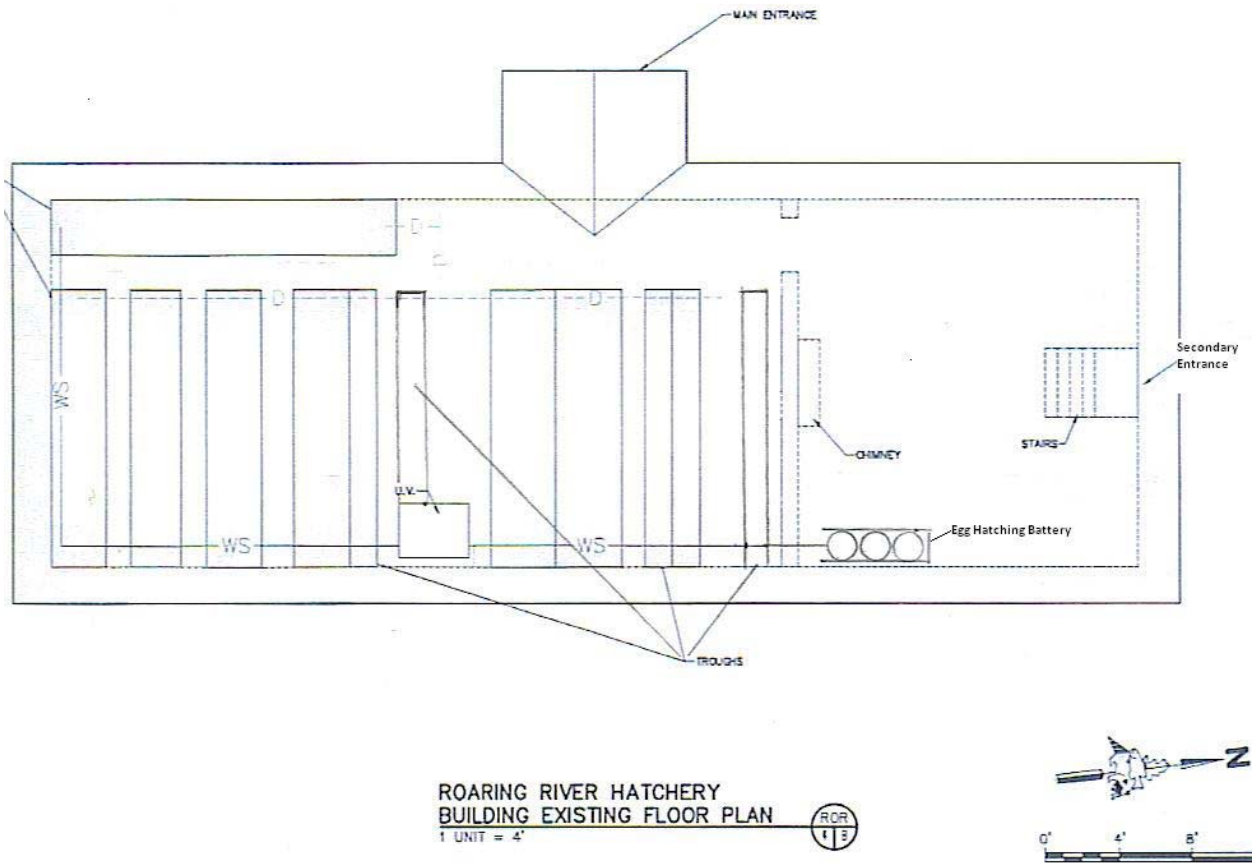


Figure 3. Floor plan of existing hatchery building.

The rearing units in the hatchery building and raceways #1 through #22 are single use systems (Figures 2 and 3). In other words, spring water flows into the head of each unit and out to the river without being reused. Raceways #23 through #40 are tiered units with water being used in three raceways before flowing out to the river (Figure 2). Many of these pools cannot be drained unless all of the pools on that system are drained. This eliminates the option to use chemical disinfectants on some outdoor raceways. Figure 1 illustrates the layout of the hatchery and life stage of fish raised in each area during normal production. Figure 2 illustrates the spring water and re-circulated water flow patterns.

Plan Components and Procedures

General Equipment Use and Cleaning

Background: Equipment and humans can transfer pathogens inside the hatchery and outside to other hatcheries. A variety of equipment is used at Roaring River Hatchery: human protective items such as waders, boots, raingear and gloves; fish handling material including nets, buckets, brushes, graders, pumps and scales; vehicles such as trucks, tanks and carts.

The use of any disinfectants at the hatchery will need to be strictly monitored since all hatchery building drains flow directly into the river. Excessive disinfectant going into the river could cause a fish kill.

General Guidelines

1. As part of this plan, hatchery staff will consult with DNR to see if Virkon® Aquatic may be approved for use on the NPDES permit. **If it cannot** be approved, then it will be essential that a hot water/steam power washer be obtained to sanitize equipment, floors and raceways.
2. In order to decrease pathogen transmission from unit to unit, additional nets and brushes will need to be purchased for the indoor troughs and outdoor rearing units. Wooden handles on existing nets will be replaced with metal or fiberglass handles. Whenever possible, equipment items will be stored at their location of use to avoid risks of contamination.
3. If Virkon® Aquatic can be approved for use, nets will be disinfected on a regular basis and always if nets must be used in different areas of the hatchery or used to pick up dead fish.
4. Unless properly disinfected, personal protective equipment will not be used if it was previously used at another hatchery or in another watershed. It is preferred that all equipment be provided on-site and only be used on-site.
5. An area north of the office building will be designated for cleaning and disinfecting equipment (Figure 1).
6. The potency of disinfectants will be tested twice a week and refreshed if needed. Table 1 provides information on commonly used disinfectants, their cost and monitoring supplies.

Vehicle Disinfection

1. Fish transfer vehicles that are used to transfer fish from other hatcheries will be washed and disinfected either on the way or at a designated site on the hatchery. Virkon® Aquatic and chlorine will be used for in conjunction with washing. If Virkon® is sprayed inside of transport tanks, it will be rinsed with freshwater after at least 10 minutes of contact time. Rinsing is important because there is the risk that the sodium chloride salt in the Virkon® may diminish the effectiveness of treatment for zebra mussel veligers.
2. Vehicles used to deliver dead fish to the composting area will also be washed and disinfected at least once per week.

Table 1. Information on common disinfectants used in aquaculture.

Products	Use/supplier	Contact Time/product	Cost
General Methods			
Hot water-power wash	General cleaning		
Steam clean	General cleaning	5 minutes	
Chemical Treatments			
Formalin 25 ppm KCL 750 ppm	Zebra mussel prevention	KCl-1 hr, then add formalin- 2 hrs = total time of 3 hrs	
Virkon® Aquatic	Western Chemical		10 lb tub= $92 10 lb (4)=$328
0.5%	General use, clothing, inside hauling tanks, equip., vehicles	10-30 min.	
1.0%	Foot bath	10 min.	replenish/check @4 days
2%	Vaccination equipment	5 min.-then rinse with water	
Argentyne, Ovadine® 50-100 ppm	Egg disinfection Argent,Western Chemical	30 min-water hardening; 10 min. after water hardening (eyed eggs)	1 gallon Ovadine® $25.95
Sodium hypochlorite (household bleach)	General cleaning	corrosive to metal, toxic to fish unless detoxified with sodium thiosulfate	
200 ppm	Vehicles	1 hour	
500 ppm	laboratory-counters/floors	10 min-1 hour	
Sodium thiosulfate	detoxify chlorine	grams thiosulfate= 2.85 x grams chlorine used	
Monitoring supplies			
Chlorine test strips	HACH	0-600 mg/l Cl2 #2890200	q=100 $17.40
	Fisher	0-200 ppm #22-479-808	q=200 $5.15
Iodine test strips	Fisher	0-100 ppm #22-307-863	q=200 $5.15
Virkon®-S test strip	Western Chemical-Antec International	see www/wchemical.com	$6.95, also provided free with each case of Virkon®

Fish Transfers

Background: About 100,000 rainbow trout on an annual basis are transferred to Roaring River Hatchery from other Missouri Department of Conservation (MDC) hatcheries or Neosho National Fish Hatchery. From March to October, trout are stocked from the hatchery into the adjacent spring branch to support the fishery within the park. Trout are also stocked into public waters and moved within the hatchery as they grow and have changing space requirements. At this time, no trout are taken from the wild. Pathogen transfer may occur during all of these activities.

Highest Risks

1. Aquatic nuisance species and pathogens (parasitic, bacterial, viral)
    a. Wild fish (unknown health status)
    b. Non-MDC hatcheries
    c. Between MDC hatcheries

An ideal **quarantine area** is "an isolated space separate from resident fish with dedicated equipment and supplies, limited foot-traffic and managed personnel and traffic flow". http://www.flsart.org/PPT/AQU-EMQ-PPT-2007-01.ppt retrieved 9/28/08

Wild Fish Transfers

Wild fish are not normally used at Roaring River Hatchery, but in the event that it is necessary to work with wild fish, the following protocol will be followed:

1. Any wild fish brought on to hatchery grounds shall be placed in isolation (in raceway #2 or #3) and not co-mingled with established hatchery populations unless they pass 6 weeks of quarantine in good health and a sample of fish from this population is found negative for VHS, IPN, IHN, whirling disease, *Aeromonas salmonicida, Yersinia ruckeri,* and *Renibacteium salmoninarum* by the Aquatic Animal Health Specialist.

2. Unhealthy wild fish or a representative sample of the population shall be evaluated and treated for parasites and diseases detected during quarantine or they will be culled.

3. Only healthy wild fish will be utilized for broodstock or other purposes.

4. All equipment used on wild fish in quarantine shall not leave the quarantine area and will be thoroughly disinfected with Virkon® Aquatic after use. Any vehicles used to transport wild fish will similarly be cleaned and disinfected after use.

5. All eggs obtained from wild fish will be disinfected with 50-100 ppm iodine (Argentyne or Ovadine®) for 15-30 minutes during water hardening. Ph shall be maintained at 7.0-7.5 . Appendix 1 provides a detailed protocol for egg disinfection.

6. If *A. salmonicida* is a concern, a second disinfection using 100 ppm for 10 minutes during the eyed stage is recommended.

Transfers from non-MDC hatcheries

1. Before a shipment of Salmonid fish shall be received from a non-MDC fish hatchery, the shipper shall submit a current fish health inspection record to the Aquatic Animal Health Specialist for review and issuance of an import permit (if necessary).

2. In addition to inspection of this report, the hatchery manager during conversations with the shipper shall ask the hatchery manager to complete and FAX or emails back a "Fish Transfer Information Sheet" that will include mortality records for the previous 15 days (Appendix 2). This sheet will help us identify in writing any other pathogens/nuisance species not specified on the health inspection record which may occur in their watershed or hatchery. This would include things like other viruses, parasites, zebra mussels, New Zealand mud snails, quagga mussels, rusty crayfish or parasitic copepods.

3. Newly imported eyed eggs shall be disinfected with 100 ppm iodine (Argentyne or Ovadine®) for 10 minutes and the shipping container shall be returned to the shipper or sprayed with 500 ppm bleach and placed in trash for disposal.

4. If fish are received, they shall be "quarantined" for 6 weeks and observed daily for signs of disease. Raceway's #2 and #3 shall be used as quarantine areas if necessary (Figure 1).

5. All equipment used during the off-loading of these fish shall be immediately disinfected after use.

Transfer of fish between MDC hatcheries

1. Three (3) days prior to the transfer of fish between MDC hatcheries, the shipping facility shall FAX or email to the receiving hatchery manager a "Fish Transfer Information Sheet" which will include a copy of the unit's mortality record for the previous 15 days, comments on the lot's general history, past chemical therapies and notes of any abnormal behaviors (Appendix 2).

3. Visibly abnormal fish will be culled prior to shipment.

4. During transport the water in the transport tank shall be treated for zebra mussel veligers per MDC policy (Appendix 3):

- 750 ppm KCL for 1 hour, then 25 ppm formalin is added for an additional 2 hours; and

- total treatment time is 3 hours.

5. When raceways are in a series, the fish will be off-loaded into the lowermost pool. When possible, this raceway should be an "isolation" unit where the fish can be monitored for and treated for post-transport disease outbreaks for at least 3 weeks before they are co-mingled with other resident fish.

6. Post-transportation stress in the fish may be reduced by providing 0.1-0.5% salt for 1-3 days after arrival.

7. Rubber boots and raingear worn during delivery of fish shall be sprayed with disinfectant before staff leave the receiving facility.

8. The transport truck shall be power washed and disinfected either en-route back to Roaring River Hatchery or at the designated hatchery cleaning site. It shall not be used for any additional loads unless it is disinfected.

Treatment of Sick Fish

Background: Disease outbreaks that occur in a rearing unit increase the risk of spreading the pathogen to other rearing units. Our goal is to isolate this "sick" unit as much as possible.

Highest Risks

1. Spreading pathogen to other rearing units on-site.

General Guidelines

1. The cause of the increased mortality shall be identified through necropsy, skin scrape, gill biopsy, clinical signs, bacterial or viral culture. It shall then be treated appropriately.

2. Units of sick fish will be considered as "quarantine areas" and specific equipment shall be dedicated for their use only. This equipment will stay at that site.

3. All equipment coming in contact with these fish and unit shall be immediately disinfected with 0.5-1% Virkon® aquatic (if authorized). It would be helpful to have a bucket of this disinfectant next to the affected raceway.

4. The fish shall not be moved/ transported off-site unless it is determined that this is necessary for their therapy. If moved to another hatchery, appropriate treatment and mortality records will be faxed or emailed to the receiving hatchery at least 3 days prior to shipment.

5. A minimum number of people shall work with these fish. In addition, staff member working in these areas should avoid handling more sensitive life stages after working with sick fish to avoid possible contamination.

6. After any contact with these fish or water (e.g. picking up mortalities, brushing raceway), staff shall wash hands with soap and water or use a hand sanitizer.

General Sanitation

Background: The maintenance of a high standard of general sanitation is a proven method for minimizing disease outbreaks in both human and veterinary practices. In our fish hatcheries, we have identified the following areas for general sanitation:

1. Handling of fish mortalities from rearing units;
2. Cleaning of rearing units between lots; and
3. Cleaning of counters and floors in fish lab and production room.

Highest Risks

1. Bacterial pathogens in dead fish are at peak levels; therefore their handling can be considered a serious mode of disease transmission.
2. Parasites and bacterial pathogens may be transmitted in water, mist spray and/or fish waste products.
3. Bacterial and viral pathogens may contaminate hands, floors and equipment in laboratory areas where sick fish are necropsied.

Fish Mortality Sanitation

1. Specific equipment (e.g., nets, buckets and vehicle) shall be designated for picking up dead fish. Buckets and nets used for this activity should be prominently labeled and not be used for any other activity.

2. A large bucket containing Virkon® Aquatic (if approved) at 1-2% should be available for soaking nets between uses. Ideally, while a net is being used another one can be soaking for at least 5 (2%) - 10 (1%) minutes before it is used again.

3. Mortalities will be counted and removed from rearing units. The total weight for daily mortalities will be weighed and recorded.

4. Mortalities shall be taken to the fish offal composting area in the park two miles from the hatchery just off F Hwy (Figures 4 and 6). Some mortalities will be frozen for use as food for captive wildlife.

5. Personnel shall wash their hands with soap and water or use hand sanitizer after collecting and disposing dead fish. Hand sanitizers shall be conveniently placed in vehicles.

6. The vehicle used to transport the dead fish shall be hosed out at the designated equipment cleaning area at least once weekly and disinfected. More frequent cleaning will be warranted if mortalities are high or there is spillage from buckets on to the bed of the vehicle.

Figure 4. Fish mortality and offal composting area, located 2 miles from the hatchery.

Cleaning Rearing Units

1. Between lots of fish, rearing units shall be dewatered completely when possible.

2. On outdoor raceways, a hot water power washer shall be used to remove organics from the sides and bottoms of the raceways. If possible, this may be followed by steam cleaning for 5 minutes. Units will then be left to dry for as long as possible.

3. Indoor tanks will be washed with a brush, sprayed with Virkon® Aquatic 0.5% (if authorized), and then allowed to dry before next use. If no chemicals are permitted, hot water or steam spraying for at least 5 minutes is recommended. Salt may be used as an abrasive.

Fish Lab

1. When sick fish are brought into the lab for necropsy, the following measures shall be taken to reduce contamination since both infected water and fluids often find their way to floors and counters.

    a. At the completion of work in the lab, the counters and floor will be sprayed or mopped with 500 ppm chlorine bleach (sodium hypochlorite) or 0.5% Virkon® Aquatic spray (if authorized).

2. For routine laboratory cleaning and disinfecting of floors 0.5% Virkon® Aquatic or 500 ppm chlorine bleach is recommended. Lower level, less corrosive disinfectants such as Roccal or Hyamin (quaternary ammonium compounds) or Lysol (phenol compound) are satisfactory (Table 2).

3. Spent scalpel blades, needles and microscope slides used during evaluations will be disposed of in a "sharps" container. Plastic loops for bacterial cultures will be disposed in a heavy-duty plastic container. Sharps and loop containers will be disinfected with 1 part bleach to 9 parts water for at least 1 hour prior to disposal in general trash.

Table 2. How to prepare a solution of household bleach (sodium hypochlorite) solution for disinfection.

$\frac{\text{(ppm Na hypochlorite desired) (gal of water) (128)}}{\text{\% of active Na hypochlorite (10,000)}}$	= Ounces of household bleach needed
e.g. $\frac{\text{(500 ppm) (1 gal)(128)}}{\text{5.25\% (10,000)}}$	= 1.2 oz of 5.25% bleach per gallon of water

Production Room for Fry

1. At all times, areas to hatch eggs and rear fry shall be kept clean.

2. A foot bath or mat using Virkon®Aquatic 1% (if authorized) shall be maintained at exits.

3. All equipment used for eggs and fry shall be designated for this area only.

4. When possible, each tank or set of tanks shall have its own net and brush. If nets or brushes are shared, they shall be appropriately disinfected between uses for 10 minutes.

5. Floors of the production room shall be mopped daily with a disinfectant if an outbreak of disease causing high mortality is occurring. Otherwise, it shall be power washed or hosed down (if power washing access is not possible) once every 2 weeks during periods of high use and mopped with a disinfectant.

Weed Cutting Boat

The weed cutting boat is used in streams to control aquatic vegetation at four MDC trout parks (Montauk, Maramec Spring, Roaring River and Bennett Spring). At different times of the year, these waterways supply water used to rear fish in the adjacent fish culture facility. At Roaring River, we are most concerned about nuisance species and unknown pathogens transferred in association with this boat use in multiple bodies of water.

Highest Risks

1. Zebra mussels
2. Nuisance aquatic plants
3. Parasitic copepods
4. Unknown pathogens carried in water

General Guidelines

1. Start with clean, dry boat.

2. After boat use, follow general cleaning protocol established for "Zebra Mussel Prevention" (Appendix 3) with the following recommendation: from among the possible disinfection methods indicated for controlling zebra mussels, power washing with hot water(>140° F for >10 sec or >176 F for >5 sec) or chlorine disinfection at 200 ppm then air drying (3-5 days) between uses at trout parks is recommended.

3. All personal equipment (waders, rain gear, rubber boots, gloves, etc.) exposed to stream water shall be sprayed with Virkon® Aquatic using a 0.5% solution before they are used again in hatchery waters.

Vaccination

Background: Vaccination is a proven method to prevent and lessen the severity of many diseases. Two vaccines, *Yersinia ruckeri* (enteric redmouth) and *Aeromonas salmonicida* (furunculosis) vaccines are available for use in salmonid fish. Both dip and injectable forms are available. At Roaring River, the enteric redmouth dip vaccine is used on fingerlings when they reach 100 fish per pound.

Highest Risks

1. Ineffective vaccination, immunity not stimulated or excessive mortality of vaccinated fish.
2. Transfer of undesirable bacteria or virus particles to other fish via contaminated needle.
    a. *Aeromonas salmonicida*
    b. *Yersinia ruckeri*
    c. Other pathogens (bacteria, viruses-unknown at time of injection).
3. Risks to humans due to accidental self-injection of vaccine.

General Guidelines

Prior to vaccination, any abnormal fish shall be culled. These fish will not be vaccinated. In addition, staff shall be cautious when vaccinating any fish that have had a previous clinical outbreak of the disease for which they will be vaccinated. In this situation, staff shall consult the product label and wait the recommended length of time before vaccinating fish (for furunculosis this is 400 degree days = #days x mean water temperature in °C [e.g. @10° C or 50° F need 40 days]).

Dip vaccines

1. Only clean sanitized dip nets shall be used to harvest fish from tanks and submerge fish in treatment containers. A new, disinfected bucket and net shall be used when the vaccine solution is changed.

2. All equipment, buckets, table, gloves, etc. shall be disinfected at the end of the day.

3. After fish are vaccinated the following information shall be obtained or recorded:

    a. Copy of the vaccine label;
    b. Date when vaccinated;
    c. Lot number and expiration date on the vaccine label (sometimes this is available as a peel off sticker);
    d. The identity of the group(s) of fish vaccinated; and
    e. Total number of fish vaccinated.

Broodstock Management

Background: Roaring River Hatchery maintains 1,000 Missouri Arlee strain rainbow trout as broodstock for egg production. These fish are spawned in January through early March and produce approximately 50% of the eggs and fry needed each year. The other eggs and fry come from Shepherd of the Hills Hatchery's Missouri strain during the months of October and November.

Highest Risks

1. Viral pathogens (VHS, IHN, IPN)
2. Bacterial pathogens (bacterial kidney disease, coldwater disease, furunculosis)

General Guidelines

1. All broodstock shall be tested for VHS, IPN, IHN, whirling disease (*Myxobolus cerebralis*), bacterial kidney disease (*Renibacterium salmoninarum*), furunculosis (*Aeromonas salmonicida*), parasitic copepods (*Salmincola* spp) and enteric redmouth (*Yersinia ruckeri*) annually per Ameircan Fisheries Society (AFS) blue book protocol.

2. At the time of spawning staff will select only apparently healthy fish for spawning. In particular, fish with poor body condition, ulcerated skin, hemorrhagic skin, darkened skin, exophthalmia, a hemorrhagic vent or very pale gills shall be culled from the breeding population.

3. Eggs will be disinfected with 50-100 ppm iodine (Argentyne or Ovadine®) for 15-30 minutes during water hardening to decrease the likelihood of pathogen transfer from infected and non-clinical carrier fish . PH should be maintained between 7.0 and 7.5 for optimal effectiveness. Appendix 1 provides a detailed protocol for Salmonid egg disinfection.

4. If eyed eggs are received from another facility for hatching or if *A. salmonicida* is a problem at the facility, the eggs will be re-disinfected with 100 ppm iodine for 10 minutes as an additional safeguard.

Public Use

Background: Public use/traffic through production areas will be closely monitored and limited to optimal viewing points, where possible. Each year, over 800,000 people visit Roaring River State Trout Park. They come from all over the world. Currently, the public is welcome to freely explore areas around our outdoor raceways, and guided tours are provided inside of hatching areas. MDC encourages educational interaction with the public at all fish hatcheries.

In addition to humans, our fish hatcheries also have wildlife visitors. Wildlife frequenting facilities include great blue herons, bald eagles, skunks, raccoons, mink, muskrats, possums, river otters and various waterfowl. Wildlife not only preys upon fish but can also transfer diseased fish from one area to another and regurgitate partially eaten food. They are recognized as biological vectors of disease.

Highest Risks

Humans

1. Although the risk is ranked as low, footwear and hands of visitors may transfer undesirable pathogens, parasites or aquatic nuisance species (e.g. ,whirling disease spores, zebra mussels) onto facilities (muddy shoes) or between rearing units (if hands are put in the water).

2. Trash or rocks thrown in water may be consumed by fish who mistake it for food.

Wildlife

1. Mechanical transfer of diseased fish or aquatic nuisance species.

General Guidelines-Humans

1. Public traffic around outdoor rearing areas is unavoidable.

2. Where possible, fish at greatest risk of disease (youngest fish or most sensitive species) will be located as far away from frequent foot traffic as possible.

3. Visitors inside of hatching areas shall be accompanied at all times and shall step into a foot bath upon entering and leaving these areas.

4. During tours the public will be instructed not to put their hands in the water.

5. A surveillance system will be installed to deter and monitor theft of fish/property or vandalism on the area.

General Guidelines-Wildlife

1. The following steps may be undertaken to discourage nuisance wildlife residence at MDC hatcheries.

    a. Dead fish will be removed from rearing units on a daily basis.

b. The practice of feeding dead fish to wildlife will be discouraged.
c. Feed storage areas will be kept clean and tidy.
d. Bird netting will be used to cover raceways with small fish in them.

General Disease and Aquatic Nuisance Species Surveillance

Background: Implementation of a surveillance program will provide a means of detecting the presence of aquatic nuisance species and pathogens for early intervention and help provide bench marks for eradication measures.

Highest Risks

1. Viral pathogens: VHS, IPN, IHN
2. Parasites: whirling disease, parasitic copepods (*Salmincola californiensis)*, Ich, trematodes, *Trichodina*, *Costia*, *Chilodonella*, *Epistylis*
3. Bacteria: *Aeromonas salmonicida, Yersinia ruckeri, Flavobacterium psychrophilum, Flavobacterium columnare, Aeromonas spp., Pseudomonas spp., Renibacterium salmoninarum, Flavobacterium branchiophilum*
4. Aquatic nuisance species: zebra mussel, New Zealand mud snails, Quagga mussels, etc.

General Guidelines

1. Good staff education is the number one preventive measure for disease surveillance. Education will provide a means for staff to recognize problems and take steps to correct them.

a. Recommend that each staff member take the Basic Fish Health class provided by MDC.

b. If there is interest, the MDC Aquatic Animal Health Specialist will be available to provide additional on-site training or refreshing of fish diagnostic techniques.

2. Artificial substrates consisting of layers of discarded plastic signs for detecting zebra mussels will be placed in the spring pool near the hatchery falls and on Roaring River near the F Hwy. bridge and will be checked every three months (Figures 5 and 6) . Directions on how to construct this monitoring device are provided in Appendix 4.

Figure 5. A sampler positive for zebra mussels (photo: Brian McKeage).

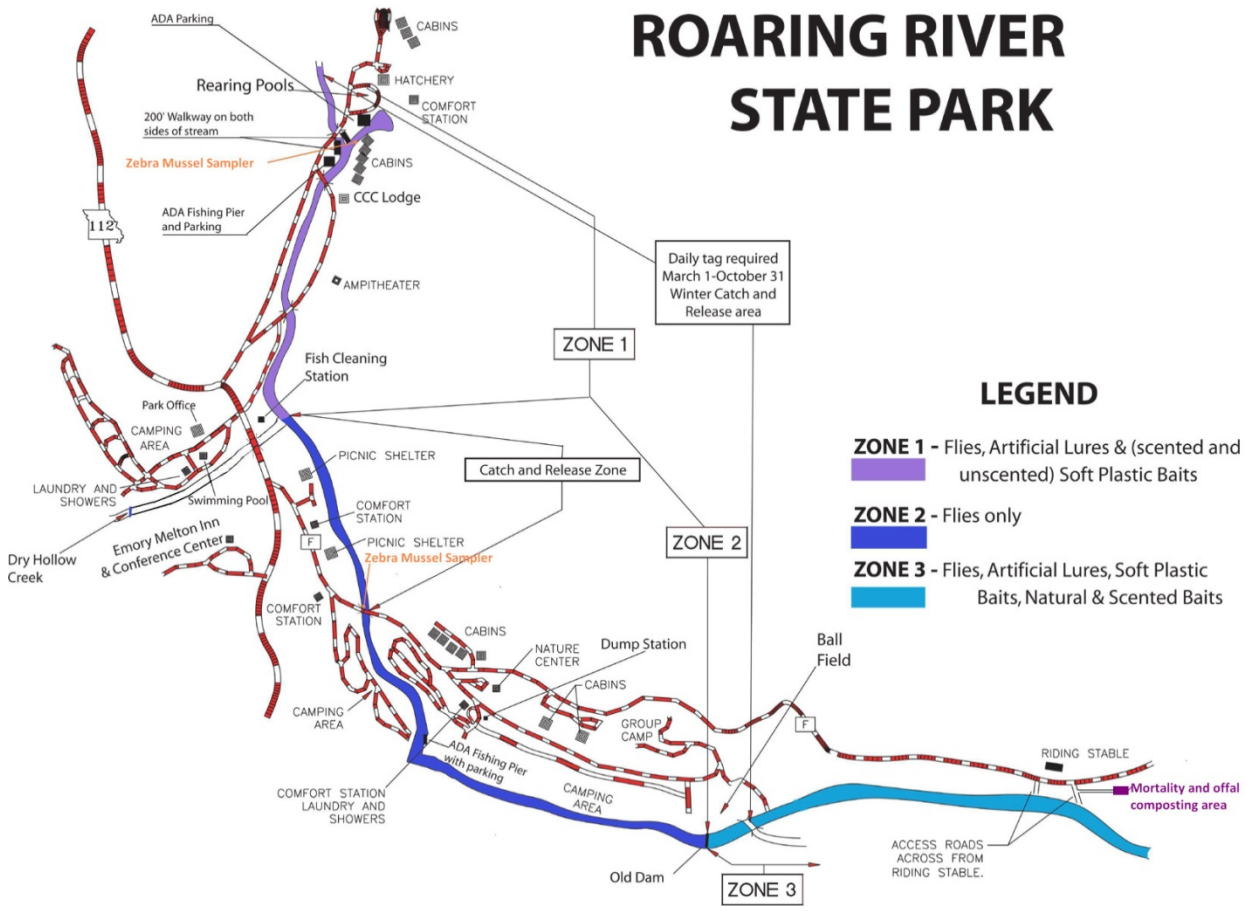


Figure 6. Location of zebra mussel samplers and fish composting site.

3. The MDC Aquatic Animal Health Specialist will conduct annual testing for viruses (IPN, IHN, and VHS), whirling disease, parasitic copepods, BKD, enteric redmouth, and furunculosis at Roaring River hatchery.

4. Fish displaying abnormal behaviors (going off feed, changes to skin color, reddened fins, increased mortalities, etc.) will be promptly evaluated (at a minimum, by general external appearance, skin scrape and gill biopsy) on-site and treated appropriately. If initial therapy is unsuccessful or if additional tests are needed which are not available on-site (e.g., bacterial culture, histopathology) they will be referred to the MDC Aquatic Animal Health Specialist for further evaluation.

5. Mortality records in writing will be maintained on a daily basis for each rearing unit. Periodically, these records will be sent to other fish hatcheries during fish transfers and will provide a means of monitoring fish health.

Environmental Concerns

Water with nitrogen gas supersaturation is often a problem at Roaring River. When excessive, it causes gas bubbles to form in the fishes' eyes, skin and gills and other blood vessels. It is a source of stress and causes mortality. Efforts will also be made to install closed packed columns inside the hatchery building on each raceway and trough to reduce nitrogen gas levels in the fry and fingerlings.

Electronic monitoring points for water levels will be connected to a telephone call system for situations when staff is not on duty. A radio alert system is currently in place to warn staff about low water levels and power loss.

It is desirable to eliminate fish from the spring pool to eliminate them as possible reservoirs of infectious agents. An effort will be made to find a way to keep fish out of the headwater of the hatchery. Any device to do this would be placed at the weir at the mouth of the spring and would have to be visually unobtrusive.

Budget Considerations

In order to implement this biosecurity plan, the following equipment items will need to be purchased. These are listed in order of priority.

1. On demand hot water heater
2. Steam cleaner
3. Extra nets, buckets, brooms and disinfectant containers
4. Extra disinfectant chemicals (if authorized)
5. Oxygen monitoring system
6. Improved water level monitors
7. Telephone alarm system
8. Video surveillance system
9. Additional chest waders for guests at the hatchery

Summary

Efforts will be made to adhere to the goals and protocols outlined in this plan. Obtaining either NPDES approval to use Virkon® Aquatic or hot water/steam cleaning equipment is a high priority for implementation of this plan because of current stringent restrictions on our discharge permits which prohibit us from using disinfectants on the area.

An on-site workshop with our staff and the Aquatic Animal Health Specialist will be set up sometime in 2010. In addition, when possible our staff will attend the fish health workshop that is conducted by the Department.

Appendix 1. HACCP for disinfection of Salmonid eggs.

HACCP Step 1 - Activity Description	
Facility: Coldwater Hatcheries	**Site**: Missouri Department of Conservation: Cold water Fish Hatcheries
Project Coordinator:	**Project Description**: Rainbow Trout Egg Collection and Disinfection
Site Manager:	
Address:	
Phone:	

Project Description **(Who, What, Where, When, How & Why)**
During Fall and Spring, rainbow trout (Oncorhynchus mykiss) are spawned at four MDC fish hatcheries (Shepherd of the Hills, Bennett Spring, Montauk and Roaring River). Each hatchery maintains its own population of rainbow trout for breeding purposes year around. Eggs are either air spawned or hand striped from females. The eggs are collected in pans then fertilized with milt from male fish. After water hardening the fertilized eggs are placed in an incubator where they develop. At the eyed stage some fish may be shipped to other facilities for hatching. Others will remain on site where they will be hatched and grown-out. At 51° F hatching will take approximately 31 days. Pathogens of greatest concern for transmission during egg collections include those viruses (VHS, IPN, IHN) and bacterias (*Aeromonas salmonicida* and *Flavobacterium psychrophilum*, *Renibacterium salmoninarum*) that can contaminate the surface of the egg or be carried within the egg.

HACCP Step 2 - Potential Hazard Identification
Vertebrates:
Invertebrates:
Plants:
Other Biologics: Viral and Bacterial Pathogens
Others:

HACCP Step 3 - Flow Diagram

Task # 1	Broodstock are gathered into holding pens.

Task # 2	Fish are anesthetized with MS-222

Task # 3	Ripe fish are selected for spawning

Task # 4	Eggs from female fish are either hand-stripped or air spawned into a stationary net to allow ovarian fluid to drain from the eggs, then the eggs are transferred to a dry stainless steel bowl.

Task # 5	Milt from males is added to the bowl along with 0.75% saline (saline will extend period of sperm motility), then the mixture is stirred.

Task # 6	Fertilized eggs are transferred to another container where distilled water is added to harden the eggs for 2 hours.

Task # 7	Eggs are rinsed in distilled water and transferred to a basket (or if placed in a bucket the eggs are periodically stirred to improve surface contact) which is then immersed in an iodine solution (50-100 ppm) for 15-30 minutes. If eggs are enumerated during this process, all measuring devices need to be disinfected before use and between lots of eggs.

Task # 8	The disinfection solution is drained and the eggs transferred to incubators.

Task # 9	Eggs are monitored daily for development, mortality and fungus. Dead

	eggs are removed.

Task # 10	Eyed eggs may be transferred to another hatchery or allowed to hatch on site where they are reared in troughs for the next several months.

HACCP Step 4 - Hazard Analysis					
Task	**Hazard**	**Probable?**	**Justification**	**Control Measures**	**CCP?**
Broodstock are gathered into holding pens.	Other Biologic: Viral and Bacterial Pathogens	Yes	Broodstock may be symptomatic or asymptomatic carriers of pathogens. Water source for hatchery is "unprotected" or contains fish so infection is possible despite tests on population.	Only a population of broodstock previously tested and found negative for VHS, IPN, IHN will be used. All equipment used to handle fish shall be disinfected after use.	Yes
Fish are anesthetized with MS-222	Other Biologic: Viral and Bacterial Pathogens	No	No pathogens are introduced in this step.		No
Ripe fish are selected for spawning	Other Biologic: Viral and Bacterial Pathogens	Yes	Some fish may be symptomatic or asymptomatic carriers of pathogens	Any apparently unhealthy fish will be culled from the breeding population.	Yes
Eggs from female fish are either hand-stripped or air spawned into a stationary net to allow ovarian fluid to drain from the eggs, then the eggs	Other Biologic: Viral and Bacterial Pathogens	Yes	Pathogens may be carried in skin mucous, sex products or inside of the egg. Other contamination could occur though use of a contaminated	Ovarian fluid will be collected and tested for viruses. Needles used for air spawning be changed frequently and disinfected between uses.	Yes

are transferred to a dry stainless steel bowl.			needle or dirty bowls.	Bowls will be clean before use and changed between egg take groups.	
Milt from males is added to the bowl along with 0.75% saline (saline will extend period of sperm motility), then the mixture is stirred.	Other Biologic: Viral and Bacterial Pathogens	Yes	Pathogens may be carried in skin mucous, milt or on surface of dirty bowls.	Symptomatic fish are culled in a previous step... Clean bowls are used in task #4.	Yes
Fertilized eggs are transferred to another container where distilled water is added to harden the eggs for 2 hours.	Other Biologic: Viral and Bacterial Pathogens	Yes	A dirty container or contaminated water may harbor pathogens. Pores of egg are open and susceptible to entry of pathogens.	Use oxygenated distilled or u/v sterilized water and make sure all containers are disinfected before use.	Yes
Eggs are rinsed in distilled water and transferred to a basket (or if placed in a bucket, the eggs are periodically stirred to improve surface contact) which is then immersed in an iodine solution	Other Biologic: Viral and Bacterial Pathogens	Yes	Improper concentration of iodine, improper pH, insufficient contact time or poor contact with the egg surface may prevent proper disinfection or cause excessive mortality of eggs	Measure iodine carefully (50-100 ppm) and spread eggs out horizontally to improve surface contact with iodine. Use a buffered approved iodine chemical (Argentyne,	Yes

(50-100 ppm) for 15-30 minutes. If eggs are enumerated during this process, all measuring devices need to be disinfected before use and between lots of eggs.				Ovadine®). Carefully time disinfection process. Ideal pH is 7-7.5. All equipment used to count eggs is disinfected before use and between lots.	
The disinfection solution is drained and the eggs are transferred to incubators	Other Biologic: Viral and Bacterial Pathogens	Yes	Disease transmission is possible if incubator is contaminated.	Disinfect incubator before use.	Yes
Eggs are monitored daily for development, mortality and fungus. Dead eggs are removed	Other Biologic: Viral, Fungal and Bacterial Pathogens	Yes	Development and survival of eggs is dependent on environmental conditions. Decay of dead eggs uses up oxygen and promotes growth of fungus.	If fungus develops, treat eggs with formalin or perox-aid. Remove dead eggs to maintain sanitary environment for developing eggs	Yes
Eyed eggs may be transferred to another hatchery or allowed to hatch on site where they are reared in troughs	Other Biologic: Viral and Bacterial Pathogens	Yes	If eggs were improperly disinfected in step 7, eggs could still be contaminated.	If eggs are shipped, receiving hatchery should soak eggs in water for 30-60 min to rehydrate them, then re-disinfect	Yes

for the next several months.				eggs for 10 min at 100 ppm iodine. This second disinfection provides additional security.	

HACCP Step 5 - HACCP Plan

Critical Control Point #1:
Task # 1: Broodstock are gathered into holding pens.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Only a population of broodstock previously tested and found negative for VHS, IPN, IHN will be used. All equipment used to handle fish shall be disinfected after use.

Limits for Control Measures:
If hatchery water supply is not "secure" fish could contract pathogen at any time after screening tests are performed; broodstock may be symptomatic or asymptomatic carriers of viruses or bacteria and may shed pathogens intermittently or have too low of a level to detect.

Monitoring: What?
Viruses (IPN, IHN, VHS), bacteria (*Yersinia ruckeri, Aeromonas salmonicida, Renibacterium salmoninarum*), and parasites (*Salmincola spp, Myxobolus cerebralis)*

Monitoring: How?
Samples of tissues are collected for viral analysis; bacteria is cultured from the posterior kidney; and cartilage is tested for Whirling Disease. Parasitic copepods are detected by visual observation.

Monitoring: Frequency?
Tests are performed annually.

Monitoring: Who?
MDC Fish Health Specialist will collect screening tests on population.

Evaluation & Corrective Actions:
Depopulate or quarantine hatchery if broodstock are found to harbor a serious pathogenic virus.

Supporting Documentation: AFS Blue book; Manual of Diagnostic Tests for Aquatic Animals OIE).

Critical Control Point #2:
Task # 3: Ripe fish are selected for spawning

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Any apparently unhealthy fish will be culled from the breeding population.

Limits for Control Measures:
Some fish are asymptomatic carriers and do not display any clinical signs of infection yet can shed the pathogen when stressed.

Monitoring: What?
Monitoring of daily health shall include detection of abnormal behaviors, mortality, physical defects or anorexia.

Monitoring: How?
Visual observation

Monitoring: Frequency?
Daily basis.

Monitoring: Who?
Hatchery staff will monitor daily health.

Evaluation & Corrective Actions:
Individual fish can be tested for virus, bacteria or parasites by Aquatic Animal Health Specialist.

Supporting Documentation:

Critical Control Point #3:

Task # 4: Eggs from female fish are either hand-stripped or air spawned into a stationary net to allow ovarian fluid to drain from the eggs, then the eggs are transferred to a dry stainless steel bowl.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Ovarian fluid will be collected and tested for viruses. Needles used for air spawning be changed frequently and disinfected between uses. Bowls will be clean before use and changed between egg take groups.

Limits for Control Measures:
Ovarian fluid from every fish cannot be checked for viruses only a representative sample from population. A proper disinfectant and contact time are needed to adequately kill virus and bacteria on needles.

Monitoring: What?
Ovarian fluid is tested for VHS, IPN, IHN.

Monitoring: How?
Ovarian fluid is collected from 60 female fish during spawning.

Monitoring: Frequency?
Annually.

Monitoring: Who?
MDC Aquatic Animal Health Specialist: ovarian fluid; Hatchery staff: spawning process.

Evaluation & Corrective Actions:
None

Supporting Documentation:

Critical Control Point #5:

Task # 5: Milt from males is added to the bowl along with 0.75% saline (saline will extend period of sperm motility), then the mixture is stirred.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Symptomatic fish are culled in a previous step. Clean bowls are used in task #4.

Limits for Control Measures:
A proper disinfectant and contact time is needed to clean a bowl.

Monitoring: What?
Cleanliness of bowl.

Monitoring: How?
Visual observation

Monitoring: Frequency?
All bowls should be clean before spawning operations begin in previous step.

Monitoring: Who?
Hatchery staff (previous step)

Evaluation & Corrective Actions:
None

Supporting Documentation: John Shrable, Daniel Abeyta, Jim McFall, David Noble. Developments in Fish Culture: Compare fertilization success when mixing eggs and milt by hand stirring versus pouring from one pan to another, USFWS, Ennis National Fish Hatchery, September 9, 1998 (unpublished).

Critical Control Point #5:

Task # 6: Fertilized eggs are transferred to another container where distilled water is added to harden the eggs for 2 hours.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Use chilled oxygenated distilled or u/v sterilized water and make sure all containers are disinfected before use.

Limits for Control Measures:
It is difficult to detect and monitor pathogens present in "untreated" water.

Monitoring: What?
Quality of water used to harden eggs.

Monitoring: How?
Visual observation and history.

Monitoring: Frequency?
Check before use.

Monitoring: Who?
Hatchery staff

Evaluation & Corrective Actions:
Locate a different water source if necessary.

Supporting Documentation:

Critical Control Point #6:

Task # 7: Eggs are rinsed in distilled water and transferred to a basket (or if placed in a bucket, the eggs are periodically stirred to improve surface contact) which is then immersed in an iodine solution (50-100 ppm) for 15-30 minutes. If eggs are enumerated during this process, all measuring devices need to be disinfected before use and between lots of eggs.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Measure iodine carefully (50-100 ppm) and spread eggs out horizontally to improve surface contact with iodine. Use a buffered approved iodine chemical (Argentyne, Ovadine®) and distilled oxygenated water. Carefully time disinfection process. Ideal pH is 7-7.5. All egg counting devices shall be disinfected with iodine prior to use and between lots of eggs.

Limits for Control Measures:

Water quality will affect activity of iodine; pH will impact egg sensitivity to iodine (survival of egg); success depends on pathogen susceptibility to iodine; and quantity and distribution of eggs will affect degree of IO surface contact.

Monitoring: What?
The concentration of iodine is monitored, the length of time of the treatment, and pH of solution are monitored

Monitoring: How?
Monitor iodine levels visually-as color fades, add more iodine; use an iodine test strip; use a stop watch for timing; use a pH meter or chemical test kit for PH. Egg continuing devices shall be visually clean before use.

Monitoring: Frequency?
Monitor constantly during disinfection. Monitor equipment before use.

Monitoring: Who?
Hatchery staff.

Evaluation & Corrective Actions:
If disinfection is not satisfactory, eggs can be re-disinfected for 15-30 minutes at 50-100 ppm after water hardening up to 5 days before hatching.

Supporting Documentation: "Iodophor disinfection of fish eggs" US Fish & Wildlife service aquatic handbook; product labels.

Critical Control Point #7:
Task # 8: The disinfection solution is drained and the eggs are transferred to incubators

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
Disinfect incubator before use.

Limits for Control Measures:
Proper disinfection technique for incubators.

Monitoring: What?
Cleanliness of incubator.

Monitoring: How?
Visual observation

Monitoring: Frequency?
Check before use.

Monitoring: Who?
Hatchery staff

Evaluation & Corrective Actions:
If dirty incubator, select another unit that is clean.

Supporting Documentation:

Critical Control Point #8:

Task # 9: Eggs are monitored daily for development, mortality and fungus. Dead eggs are removed

Significant Hazards:
Other Biologic: Viral, Fungal and Bacterial Pathogens

Control Measures:
If fungus develops, treat them with formalin or Perox-aid. Remove dead eggs to maintain sanitary environment for developing eggs

Limits for Control Measures:
Amount of available labor will dictate how frequent dead eggs can be removed; water quality will also impact success of egg development.

Monitoring: What?
Staff will monitor the color of the egg for detecting viability. White or black eggs are dead. Fuzz indicates fungus.

Monitoring: How?
Visual observation.

Monitoring: Frequency?
Daily basis during egg development.

Monitoring: Who?
Hatchery staff.

Evaluation & Corrective Actions:
None other than destroying the unit of infected eggs.

Supporting Documentation:

Critical Control Point #9:

Task # 10: Eyed eggs may be transferred to another hatchery or allowed to hatch on-site where they will be reared in troughs for the next several months.

Significant Hazards:
Other Biologic: Viral and Bacterial Pathogens

Control Measures:
If eggs are shipped, receiving hatchery should soak eggs in water for 30-60 min to rehydrate the eggs, then re-disinfect eggs for 10 min at 100 ppm iodine. This second disinfection provides additional security.

Limits for Control Measures:
If initial disinfection was not successful or eggs were contaminated in hatchery water,

eggs may still harbor pathogens. Iodine disinfection should not be done within 5 days of hatching since this may kill the developing embryo or cause premature hatching
Monitoring: What? Staff will monitor iodine treatment as in task #7, critical control point # 6.
Monitoring: How? See critical control point #6
Monitoring: Frequency? See critical control point #6.
Monitoring: Who? Hatchery staff of receiving facility
Evaluation & Corrective Actions: None
Supporting Documentation:

Facility: Coldwater Hatcheries	**Activity**: Rainbow Trout Egg Collection and Disinfection
Address:	
Signature:	**Date**:

Facility

Site Missouri Department of Conservation: Cold water Fish Hatcheries

Coordinator

Manager

Address ,

☐ **Task # 1: Broodstock are gathered into holding pens. CRITICAL CONTROL POINT**

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Only a population of broodstock previously tested and found negative for VHS, IPN, IHN will be used. All equipment used to handle fish shall be disinfected after use.
- ☐ Control limits were maintained
  Control Limits: If hatchery water supply is not "secure" fish could contract pathogen at any time after screening tests are performed; broodstock may be symptomatic or asymptomatic carriers of viruses or bacteria and may shed pathogens intermittently or have too low of a level to detect..
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: Depopulate or quarantine hatchery if broodstock are found to harbor a serious pathogenic virus.

☐ **Task # 2: Fish are anesthetized with MS-222**

☐ **Task # 3: Ripe fish are selected for spawning CRITICAL CONTROL POINT**

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens

- ☐ Control measures were implemented
  Control Measures: Any apparently unhealthy fish will be culled from the breeding population.
- ☐ Control limits were maintained
  Control Limits: Some fish are asymptomatic carriers and do not display any clinical signs of infection yet can shed the pathogen when stressed.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: Individual fish can be tested for virus, bacteria or parasites by Aquatic Animal Health Specialist.

☐ **Task # 4: Eggs from female fish are either hand-stripped or air spawned into a stationary net to allow ovarian fluid to drain from the eggs, then the eggs are transferred to a dry stainless steel bowl.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Ovarian fluid will be collected and tested for viruses. Needles used for air spawning be changed frequently and disinfected between uses. Bowls will be clean before use and changed between egg take groups.
- ☐ Control limits were maintained
  Control Limits: Ovarian fluid from every fish cannot be checked for viruses only a representative sample from population. A proper disinfectant and contact time are needed to adequately kill virus and bacteria on needles.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: None

☐ **Task # 5: Milt from males is added to the bowl along with 0.75% saline (saline will extend period of sperm motility), then the mixture is stirred.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Symptomatic fish are culled in a previous step. Clean bowls are used in task #4.
- ☐ Control limits were maintained
  Control Limits: A proper disinfectant and contact time is needed to clean a bowl.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: None

☐ **Task # 6: Fertilized eggs are transferred to another container where distilled water is added to harden the eggs for 2 hours.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Use oxygenated distilled or u/v sterilized water and make sure all containers are disinfected before use.
- ☐ Control limits were maintained
  Control Limits: It is difficult to detect and monitor pathogens present in "untreated" water.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: Locate a different water source if necessary.

☐ **Task # 7: Eggs are rinsed in distilled water and transferred to a basket (or if placed in a bucket, the eggs are periodically stirred to improve surface contact) which is then immersed in an iodine solution (50-100 ppm) for 15-30 minutes. If eggs are enumerated during this process, all measuring devices need to be disinfected before use and between lots of eggs.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: Measure iodine carefully (50-100 ppm) and spread eggs out horizontally to improve surface contact with iodine. Use a buffered ,approved iodine chemicals (Argentyne, Ovadine®). Carefully time disinfection process. Ideal pH is 7-7.5.
- ☐ Control limits were maintained
  Control Limits: Water quality will affect activity of iodine; pH will impact egg sensitivity to iodine (survival of egg); susceptibility of pathogen to iodine; and quantity and distribution of eggs will affect degree of IO surface contact.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: If disinfection is not satisfactory, eggs can be re-disinfected for 10-30 minutes at 50-100 ppm after water hardening up to 5 days before hatching.

☐ **Task # 8: The disinfection solution is drained and the eggs are transferred to incubators.**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented

Control Measures: Disinfect incubator before use.

- ☐ Control limits were maintained
  Control Limits: Proper disinfection technique for incubators.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: If dirty incubator, select another unit that is clean.

☐ **Task # 9: Eggs are monitored daily for development, mortality and fungus. Dead eggs are removed**
CRITICAL CONTROL POINT

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral, Fungal and Bacterial Pathogens
- ☐ Control measures were implemented
  Control Measures: If fungus develops, treat them with formalin or Perox-aid. Remove dead eggs to maintain sanitary environment for developing eggs
- ☐ Control limits were maintained
  Control Limits: Amount of available labor will dictate how frequent dead eggs can be removed; water quality will also impact success of egg development.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: None other than destroying the unit of infected eggs.

☐ **Task # 10: Eyed eggs may be transferred to another hatchery or allowed to hatch on site where they are reared in troughs for the next several months.**

- ☐ Hazards were contained
  Hazards: Other Biologic: Viral and Bacterial Pathogens
- ☐ Control measures were implemented

Control Measures: If eggs are shipped, receiving hatchery should soak eggs in water for 30-60 min, and then re-disinfect eggs for 10 min at 100 ppm iodine. This second disinfection provides additional security.

- ☐ Control limits were maintained
  Control Limits: If initial disinfection was not successful or eggs were contaminated in hatchery water, eggs may still harbor pathogens. Iodine disinfection should not be done within 5 days of hatching since this may kill the developing embryo or cause premature hatching.
- ☐ Corrective actions were (performed if necessary)
  Corrective Actions: None

Appendix 2. **Fish Transfer Information Sheet**

(Submit via email at least 3 days prior to shipment)

Today's date: _____

Anticipated shipment date: _____

From: ☐ Bennett ☐ Maramec ☐ Montauk ☐ Roaring River ☐ Shepherd ☐ Other: ____

To: ☐ Bennett ☐ Maramec ☐ Montauk ☐ Roaring River ☐ Shepherd ☐ Other: ____

Lot Designation: _____ From Raceway/Unit: _____

Lot History

Mortality record for last 15 days provided (see page 2)? ☐ Yes ☐ No
Fish taken off feed 3 days prior to shipment? ☐ Yes ☐ No

The following aquatic nuisance species occur in this hatchery's watershed:
☐ Zebra mussels ☐ Parasitic copepods ☐ Rusty crayfish ☐ Quagga mussels
☐ New Zealand mud snails ☐ Other: _____

General Health within last 30 days: ☐ Excellent ☐ Good ☐ Fair ☐ Poor
(Basis of this classification ☐ Mortality record ☐ Observation/feed intake Necropsy: gills, skin, organs)

If fair or poor: what problems were observed or suspected? _____
Any chronic problems? _____

Therapeutics used in last 30 days:

Therapeutic Used	Yes √	Date Treated	Why treated?	Results or Comments (cured problem; partial, little or no improvement)
None	☐			
Aquaflor	☐			
Chloramine-T	☐			
Copper sulfate	☐			
Formalin	☐			
Immersion OTC	☐			
MS-222	☐			
Oxytetracycline	☐			
Perox-Aid	☐			
Romet	☐			
Salt	☐			
Vinegar	☐			

Mortality Record Previous 15 days

Date		
Month	Day	Daily Mortalities

Appendix 3. MDC Zebral mussle prevention policy.

ZEBRA MUSSEL PREVENTION

POLICY

The Missouri Department of Conservation will work to prevent the spread of zebra mussels from infested waters to uninfested waters.

est. 10/05

PROCEDURES

● RESOURCE THREAT

Zebra mussels can clog power plants, industrial and public drinking water intakes, foul boat hulls, decimate populations of freshwater mussels and other native aquatic organisms, impact fisheries and disrupt aquatic ecosystem functions. Economic impacts of zebra mussels in North America are estimated to be in the billions of dollars. Because of the ease with which microscopic larval zebra mussels may be transported by the public, it may take several years to detect an infestation. Avoiding known infested areas, or staging equipment use such that waters known, or suspected to be infested, are visited last, will help prevent the spread of zebra mussels. However, boats, equipment, and gear must be decontaminated prior to use in different waters. Personnel will take reasonable precautions to avoid exposure of equipment, facilities, and other waters to zebra mussels.

● PUBLIC OUTREACH AND EDUCATION

Increased public outreach and education will enhance understanding of the potential problems associated with zebra mussels and the measures that may help deter their expansion. Signs should be posted at all MDC owned and managed boat ramps highlighting the potential problems associated with zebra mussels. Information should be distributed through our state, federal and non-governmental agency partners, MDC managed waterfowl areas, trapping associates, sport fishing groups, marinas, lake associations, Department offices and Nature Centers, media outlets and to other water users in Missouri.

● EQUIPMENT DECONTAMINATION PROCEDURES

Appropriate safeguards to prevent the transfer of zebra mussels from one waterbody to another are mandatory and include inspection, treatment, and, if possible, avoidance. The following steps detail equipment decontamination procedures:

1. Thoroughly inspect boats (hulls, drive units, trim plates, transducers), trailers and components (rollers, bunk boards, axles, etc.), equipment (i.e., water pumps, hatchery equipment, siphons, nets, ropes, traps, etc.), and machinery (tractors, bulldozers, etc.) for adult zebra mussels. Pay close attention to nooks, crannies and other inconspicuous places (i.e., around the motor housing, trim tabs, and water intake screens, or pump fittings). All trash, mud, vegetation, and suspected zebra mussels should be removed and properly disposed of in the trash. Immediately report suspected occurrences of zebra mussels to the Invasive Species Coordinator.

2. Carpeted bunks and runners on existing boat trailers should be replaced with poly,

plastic or wooden bunks as soon as practical; boat trailers regularly moved between known zebra mussel infested waters and other waters should have carpeted bunks and runners replaced immediately. As available, future boat trailers should be purchased with poly/plastic/wooden bunks.

3. All water should be drained from boats, trailers, motors, live wells, bilges, transom wells, holding tanks and live wells, water pumps, pipes, and other equipment prior to leaving a waterway. Pay particular attention to boat hulls under installed decking. Drain as much water as possible from equipment such as lower motor units and portable pumps.

4. Any boat, trailer, tank, equipment, machinery, gear, or net transferred from one body of water into a different body of water or from known infested waters to potentially infested waters must be decontaminated using one of the treatments in Table 1 prior to being used in a new body of water. Equipment decontamination procedures should be completed when moving equipment from infested areas of a water body to uninfested areas of the same water body. If boats, nets, and other equipment are only used in one body of water, cleaning between uses is not necessary, but these boats, nets, and other equipment MUST be clearly labeled for use in that body of water ONLY. Periodic cleaning and decontamination (i.e., during winterization or other maintenance) should be conducted to prevent costly repairs. If management or research activities require this equipment to be moved in the future, decontamination procedures will be implemented.

● HATCHERY PRECAUTIONS

Best management practices should be used to protect equipment and facilities and to reduce the opportunity for the spread of zebra mussels to uninfested areas. Introductions of zebra mussels into MDC fish hatcheries or water supply sources would have devastating impacts upon hatchery infrastructure. If infested, hatcheries would then be a possible mechanism for transporting the organisms to uninfested waters. Therefore, the following precautionary measures will be enacted by MDC fish hatcheries:

1. All attempts will be made to secure fish from sources known to be free of zebra mussels (veligers and adults) (see map at http://intranet/Documents/17407.pdf)

2. All fish and eggs exposed to surface water coming into or leaving any of MDC's hatcheries or other facilities and any fish procured through contract or other means from outside sources must be treated during transportation using one of the treatments in Table 3. The only exception will be for fish that are stocked into the same water supply that is used by the hatchery (e.g., trout stocked in Bennett Spring branch by Bennett Spring Hatchery staff) and for selected species of conservation concern.

3. Specific limitations may be applied to native mussel and hellbender culture, and other species of conservation concern, on a case-by-case basis.

4. Some species or life stages of fish or other aquatic organisms may be less tolerant of chemical treatments. For these species or life stages whose chemical tolerances are unknown, bioassays must be performed prior to large scale use of the prescribed treatments listed below. Until these bioassays are conducted, brood stock of these species will only be obtained from waters known to be free of zebra mussels.

Table 1. Zebra Mussel Disinfectants and Usage Guidelines for Boats and Equipment

Disinfectant	**Concentration**	**Contact Time**	**Usage Guidelines, Safety Precautions, Drawbacks**
Vinegar	100%	20 min	Use appropriate personal protective equipment (PPE) and caution. Stay upwind of the spray. Is corrosive to metal and toxic to fish at this concentration, so thoroughly rinse with tap water or water from the next lake or river after disinfection. Ensure that solution does not run-off directly into waterways.
Chlorine	200 ppm	10 min	Use appropriate PPE and caution. Stay upwind of the spray. Is corrosive to metal and rubber and toxic to fish at this concentration, so neutralize with 800 ppm sodium thiosulfate and rinse thoroughly with tap water or water from the next lake or river. Ensure that solution does not run-off directly into waterways.
Power wash with hot water	>104° F	20 min	Use appropriate PPE and caution when using hot water due to possibility of burns/scalding. Temperature and contact times are crucial, as efficiency is weather dependent. Most effective when used in conjunction with air drying (see below). Power wash with hot water, including thoroughly flushing lower motor unit.
Freezing	<32° F	24 hrs	Boats, gear, and equipment should be thoroughly frozen. Ambient air temperature should remain below freezing for the entire contact time. No safety precautions.
Air drying	N/A	3-5 days in hot sun 48 hrs in hot sun	Must dry completely to be effective. Most effective when used in conjunction with hot water (see above). To be used for small nets, gear, pumps, etc., *ONLY AFTER* power washing with hot (104°) water for appropriate contact time.

Salt Bath	1%	24 hrs	Due to the long contact time, may only be used as a bath solution and not sprayed. To be used only for pieces of equipment, gear, and nets that can be completely immersed in the solution.

Table 2. Disinfectant Amounts to Make Needed Concentrations					
Disinfectant	**1 gallon**	**2 gallons**	**5 gallons**	**20 gallons**	**100 gallons**
100% Vinegar	1 gal	2 gal	5 gal	20 gal	100 gal
200 ppm Chlorine (household bleach, 5.25% Chlorine)	0.5 ounce (15 ml)	1.0 ounce (30 ml)	2.5 ounces (75 ml)	11.0 ounces (300 ml)	6 1/3 cups (1.5 L)
200 ppm Chlorine (HTH granular)	0.04 ounce (1.2 g)	0.08 ounce (2.4 g)	0.2 ounce (6 g)	0.8 ounce (24 g)	4.2 ounces (120 g)
800 ppm Sodium Thiosulfate	0.1 ounce (3 g)	0.2 ounce (6 g)	0.5 ounce (15 g)	2.1 ounces (60 g)	10.6 ounces (300 g)
1% Salt Bath (as NaCl)	1/8 cup	1/4 cup	2/3 cup	2 2/3 cups	13 1/3 cups

Notes:

1. Air drying and hot water are most effective when used in conjunction with each other because their effectiveness is highly dependent upon ambient temperatures and contact times. As needed, hot water wash units should be made available at selected Department facilities.
2. Household bleach (5.25% chlorine) and vinegar can be purchased from grocery or convenience stores. HTH granular chlorine (70% calcium hypochlorite) and Sodium Thiosulfate can be purchased at pool supply stores or chemical companies.
3. All bilges and hidden areas under boat decks must be thoroughly treated as described above.
4. Source: WI DNR (2007) *Equipment Disinfection Protocol for Invasive Species and Viruses*.

Table 3. Hatchery/Fish/Aquatic Organism Zebra Mussel Treatments and Usage Guidelines			
Treatment	**Concentration**	**Contact Time**	**Usage Guidelines/Comments**
NaCl	20,000 ppm	2 hrs	Used for striped bass only. Treatment conducted during transport.
KCl/formalin	750 ppm KCl 25 ppm formalin	1 hr 2 hrs	Used for all other fish species and eggs. Fish and hauling water are pretreated for 1 hour with 750 ppm KCl, followed by a 2 hour treatment with 25 ppm formalin during transport. *DO NOT* treat fish with NaCl to counteract shock, as this decreases the effectiveness of the treatment.

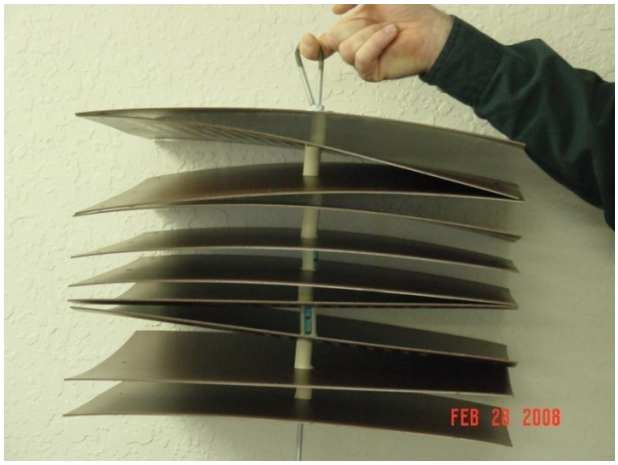
Notes:

1. All fish, including those used in aquaria at nature centers, fairs, etc., are to be treated for zebra mussels while in transit.
2. Treatment concentrations and contact times that are currently exceeded during normal aquaculture operations (e.g., egg hardening and shipping) should be considered effective.
3. Some species or life stages of fish or other aquatic organisms may be less tolerant of chemical treatments. For these species or life stages whose chemical tolerances are unknown, bioassays must be performed prior to large scale use of the treatments listed above.
4. For species with known intolerances to recommended zebra mussel treatments, modifications of hatchery assignments, increased use of well water, UV treatment, sand filtration, and other system modifications or treatment/avoidance measures may be needed and should be considered on a case-by-case basis with the involvement and approval of Division Chiefs and the Invasive Species Coordinator.
5. Sources: IA DNR *Fairport Fish Hatchery ANS-HACCP*, Edwards *et al*. 2000.

Appendix 4. Directions for construction of a zebra mussel monitoring device from Brian McKeage, email: 3/23/09.

They are pretty simple to make. All you need is 10 old Department plastic signs, a piece of 5/16" all thread with a loop bent in the top, 1" pieces of ½"cpvc pipe and a nut and washer. Drill a hole through the signs, place the signs on the all thread with a loop, put the cpvc pipe pieces in to space out the signs and finish by putting the nut and washer on after the last sign. You can then tie a rope to the loop and hang the monitor up in the water body.

Scott has the picture of the monitor before we put it in the water.



Thanks,

Brian McKeage

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